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Determination of the nutrient composition and associated fungi of malus domestica sold in port harcourt metropolis

Chuku EC[1], Emiri UN[2]
[1] Depratment of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria
[2] Department of Agricultural Education, ISAAC Jasper College of Education, Sagbama, Bayelsa State, Nigeria

Abstract
Studies on the assessment of the nutrient composition and associated fungi of *Malus domestica* were carried out in the Department of Plant Science and Biotechnolgy laboratory, Rivers State University. Proximate parameters assessed were moisture, ash, fibre, lipid, carbohydrate and protein. Increased values for fibre, carbohydrate and protein were seen in the healthy samples while moisture, ash and lipid were higher in the spoilt fruit samples. Findings from the mineral and vitamin studies showed that both healthy and spoilt samples of *M. domestica* had equal values for calcium (12.5± 0.003), potassium (8.5± 0.004), iron (0.38± 0.001) and vitamin C (75± 0.001). However, magnesium, phosphorus and sodium had higher values for the healthy fruit samples. Notwithstanding, phytochemical analysis showed that the healthy fruits of *M. domestica* possesed tannin, saponin, oxalate and cyanogenic glycoside in appreciable amounts. Five fungal organisms with varying incidence were isolated viz: *Botrytis cinerea*, *Fusarium oxysporum*, *Aspergillus flavus*, *Cryptococcus neoformans* and *Penicillium italicum*. The highest percentage incidence was observed for *B. cinerea* (50± 0.002). This was seconded by *F. oxysporum* (20± 0.004) while *A. flavus*, *C. neoformans* and *P. italicum* recorded the least fungal incidence.

Keywords: nutrient composition, fungi and *Malus domestica*

Introduction
Apple plant scientifically known as *Malus domestica* is deciduous and belongs to the family Rosaceae. Owing its origin from central Asia, the genus malus possesesseveral species (Phipps, 1990; Muhammad *et al*., 2013) [17, 13]. The exotic plant is so cheerished for its delicious fruit and has an increased comsumption rate (Oranusi and Braide, 2012) [16]. The tree attains a height range of 1.8 to 9.1 meters, and is influenced by trimming and rootstock. However, alternately arranged leaves which are dark green and white flowers (with tinge of pink) which usually appear during spring are characteristics of *M. domestica* (Lauri *et al*., 2006) [11]. Colour and size variations exist among the fruits which are harvested during summer or autum and each fruit may contain 1 to 3 seeds (Vaibhav *et al*., 2012; Coart *et al*., 2006) [21, 3].
M. domestica, though not grown and cultivated within the tropic are cheerised by Africans including Nigerians as its fruit is endowned with vital nutritional components. The fruits and seeds have also been noted to be of importance in the industry for the production of wine and oil respectively (Yu *et al*., 2007; Gerhauser, 2008) [22, 4].
Proximate assesment of *M. domestica* fruits by early researchers revealed the presence of moisture, ash, fiber, protein, carbohydrate and fat at various appreciable amounts (Muhammad *et al*., 2013; Vaibhav *et al*., 2012) [13, 21]. Nontheless, the fruits also contain several minerals and vitamins of various concentrations. Calcium, sodium, potassium, phosphorus, magnesium, iron, zinc and vitamins A, E, K and C were all implicated by early reseachers to be present in *M. domestica* fruits (Nava *et al*., 2018; Horsley *et al*., 2014) [15, 7]. The presence of these minerals was also supported by the research of Nachtigall and Dechen (2016) [14], as the above mentioned minerals were reported to be found in the fruit of *M. domestica*. They also found out that the level of nutrient accumulation increased with fruit size.
More so, earlier investigation has revealed the presence of phytochemical and phenolic compounds in *M. domestica* at various concentrations. The presence of these components have lead to the great medicinal importance attached to apple fruit and juice (Hyson, 2011) [8]. The availability of these components have also been reported to provide anticarcinogenic, antimicrobial, antioxidant and antiinflammatory properties (Fratianni *et al*., 2007; Boyer and Liu, 2004; Gerhauser, 2008) [5, 2, 4].
However, the consumption and storage of this important fruit within Rivers State in Nigeria is seriouly affected by spoilage organisms. Microorganisms including bacteria and fungi have been implicated to cause this menace on apple fruit as well as other important edible fruits and vegetables (Udoh *et at*., 2015; Ayanda *et al*., 2013; Leff and Fierer, 2013) [20, 1, 12]. Futhermore, fungi organisms isolated from apple fruits by early researchers include: *Rhizopus spp*, *Penicillium spp*, *Aspergillus niger*, *A. Flavus*, *Fusarium spp*, *Mucor spp*, *S. cerevisiae*, *Candida spp* and *Botrytis spp* (Oranusi & Braide, 2012; Scheper *et al*., 2007) [16, 18]. The activities of these organisms have affected the quality of *M. domestica* fruits sold in Port Harcourt metropolis and indirectly influenced the income of the vendors.
It is on this light that this research wascarried outto evaluate the nutritional quality of *M. domestica* fruits sold in Port Harcourt and its associated fungi organisms.

Materials and Methods
Sample Collection
Samples of healthy fruits of *M. Domestica* and partially

rotted fruits were bought from the Fruit Garden Market at D. Line Diobu Port Harcourt and brought to the Department of Plant Science and Biotechnology and sent to the Plant Pathology Laboratory for futher studies.

Mycological studies

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120ºC for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud Dextrose Agar prepared in a conical flask using the standard method. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask containing the mycological medium was autoclaved at 121º C and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40 º C and dispensed into Petri dishes at 15mls per plate and allowed to further cool and solidify.

Isolation of fungi from partially rotted *M. domestica* fruits

One gram of samples showing visible signs of spoilage by Moulds was cut from the healthy portions of the fruits up to the points where rot had established and inoculated onto Sabouraud Dextrose Agar in Petri dishes onto which ampicillin was added to hinder the growth of bacteria in triplicate. The inoculated plates were incubated for 5 days at ambient temperature of 25º C ± 3º C (Baudoni, 1988, Chuku, 2009, Samson *et al*, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates were obtained after a series of isolations**.**

Identification of fungal organisms from *M. domestica*

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically using both the low and high power objective. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson *et al*, 1981 and Olds, 1983).

Pathogenicity studies

Pathogenicity studies was carried out on *M. domestica* to check if the fungi isolated from the rotted fruits were capable of causing spoilage on healthy fruits samples. The methods of (Agrios, 2005, and Trigiano, 2004) was basically followed. The fungal isolates were introduced into healthy fruits and observed for seven days. The set up was monitored regularly for growth.

Determination of nutrient components of healthy fruits of *M. domestica*.

Healthy fruit samples of M. domestica were sent to the Food Science and Technology Laboratory for the determination of nutrient composition. The methods of AOAC (2005) was used for the analysis.

Results and Discussions

Table 1: Proximate composition of healthy and spoilt *M. domestica* fruits

Parameters	Healthy (%)	Spoilt (%)
Moisture	90.2± 0.006	90.4± 0.001
Ash	0.96± 0.001	0.97± 0.004
Fibre	0.20±0.002	0.15± 0.001
Lipid	0.05± 0.001	0.06± 0.005
Carbohydrate	6.29± 0.003	6.22± 0.002
Protein	2.30±0.002	2.20±0.003

Table 2: Minerals and vitamin composition of healthy and spoilt M. domestica fruits

Parameters	Healthy (mg/100g)	Spoilt (mg/100g)
Calcium	12.5± 0.003	12.5± 0.003
Iron	0.38± 0.001	0.38±0.001
Magnesium	6.7±0.002	5.5± 0.004
Potassium	8.5± 0.004	8.5± 0.004
Phosphorus	11.2±0.003	11.1±0.002
Sodium	2.8±0.001	2.7± 0.005
Vitamin C	75±0.001	75±0.001

Table 3: Phytochemical composition of *M. Domestica*

Parameter	Percentage occurrence (%)
Tannin	0.2± 0.004
Saponin	0.11± 0.001
Oxalates	0.30± 0.002
Cyanogenic glycoside	1.4± 0.001

Table 4: Fungi isolates and percentage incidence

Isolates	Percentage incidence (%)
Botrytis cinerea	50± 0.002
Fusarium oxysporum	20± 0.004
Aspergillus flavus	10± 0.001
Cryptococcus neoformans	10± 0.003
Penicillium italicum	10±0.001

Table 1. reveals the proximate result of *M. domestica*. Moisture content was lower in the healthy fruits (90.2±0.006) compared to the spoilt (90.4± 0.001). While (0.96±0.001) forash was recorded for healthy *M. domestica* fruits, (0.97±0.004) was seen for the spoilt. The fibre contents recorded were(0.20± 0.002) and (0.15±0.001) for the healthy and spoilt samples respectively. Meanwhile, lipid recorded (0.05±0.001) for the good samples and (0.06±0.005)for the spoilt samples. Carbohydrate recorded (6.29±0.003) for the healthy samples of *M. domestica* while (6.22±0.002) was given for the spoilt samples. The protein values recorded were (2.30± 0.002) and (2.20±0.003) for healthy and spoilt samples of *M. domestica* respectively. Generally, while higher values for fibre, carbohydrate and protein were recorded for the healthy samples, other parameters assessed were higher for the spoilt samples. The values recorded for proximate composition in this study is in line with those reported by early reseachers. However, the values in this study are lower than those reported by Muhammad *et al*., (2013) [13] for *M. domestica* except for moisture and protein which were lower compared to those assessed in this study. Also, the values reported by Vaibhav *et al*., (2012) [21] are higher for carbohydrateand fibre but lower for potein when compared to its equivalents in this study.

The minerals and vitamin results presented in Table 2. showed that calcium, potassium, iron and vitamin C had equal values for both healthy and spoilt fruit samples of *M. domestica*. Nevertheless, higher values of magnesium (6.7±0.002 mg/100g), phosphorus (11.2±0.003mg/100g) and sodium (2.8± 0.001 mg/100g) were recorded for the healthy fruits compared to their equivalent parameters which had lower values for the spoilt fruit samples. The above result is supported by early findings as all assessed parameters have been implicated to be present in *M. domestica* fruit. However, the findings of Horsley *et al*., (2014) [7] implicated higher values for potassium, magnesium and phosphorus compared to the results obtained in this study. However, the calcium value in this study is higher than that reported in their research. Also, the report on the mineral composition of *M. domestica* by Muhammad *et al*., (2013) [13] showed higher values for all the mineral parameters recorded inthis study. The vitamin C value in this study is higher than those reported by Vaibhav *et al*., (2012) [21] and Muhammad *et al*., (2013) [13]. The presence of these nutritional components are essential for the effective functioning of a biological system (Smolin and Grosvenor, 2000) [19].

In addition, the result from phytochemical study as presented in Table 3. revealed the presence of tannin,saponin, oxalates and cyanogenic glycocide in appreciable amounts. This finding is in agreement with early reseach carried out, as several other phytochemicals have been recorded in *M. domestica* fruits (He and Liu, 2007) [6]. More so, these phytochemicals play vital roles in the human health and posses potentials to fight against invading microorganisms (Fratianni *et al*., 2007) [5].

Table 4. shows the various fungal organisms isolated and their respective percentage incidence. Five fungi were isolated and they are *Botrytis cinerea*, *Fusarium oxysporum*, *Aspergillus flavus*, *Cryptococcus neoformans* and *Penicillium italicum*. The highest incidence was observed *for B. cinerea* (50 ± 0.002) and was followed by (20 ±0.004) for *F. oxysporum*. Meanwhile, the other three isolates had equal incidence. The fungi organisms isolated in this study have been reported by early reseachers to cause spoilage. The current isolates agrees with Juhnevica *et al*., (2011) [9] as they isolated *Penicillium spp*, *Botrytis spp* and *Aspergillus spp*. Ayanda *et al*., (2013) [1] also implicated *A. flavus* and *A. niger* in the deterioration of *M. domestica* fruits. The research of Oranusi and Braide, (2012) [16] also supports the isolates of this current study as all the above isolated organisms in Table 4. were reported, except for *Botrytis*, to be responsible for the spoilage of *M. domestica fruits*. The presence of these organisms on and in ready to eat fruits of *M. domestica*makes it unhealthy and not safe for consumption, as they are capable of causing several diseases due to the production of mycotoxins (Katherine *et al*., 2006). It is however very important to note that *M. domestica* is an exotic fruit that does not thrive in the tropical environment and as such imported into various countries where they are not grown. The use of preservatives to keep the fruits fresh for the lenght of time while on transit and the environmental differences may have affected the nutient composition of these fruits. The fungal isolates from the fruits may also have been influenced by the factors mentioned above. It is therefore suggested that this work be repeated at various geographical zones in Nigeria.

Conclusion

It is a general fact that most fruits are rich in minerals and essential micro and macro nutrients of which*M. domestica* is not an exemption. However, improper handling of fruits during storage and processing would made them unsafe for consumption as they are prone to fungal contamination. Hence, appropraite hygeinic measure should be adhered to by vendors during packaging and sales. Consumers should aslo endeavour to wash fruits properly before consumption.

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